One Health Case Study of Chikungunya: An Emerging Disease

STUDENT GUIDE

Renee Prater, DVM, MS, Ph.D.
Teresa R. Johnson, MS, Ph.D.
Alexis M. Stoner, MPH
Matthew D. Cannon, DO
Nammalwar Sriranganathan B.V.Sc., M.V.Sc., Ph.D., ACVM

OBJECTIVES

1. Define the pathogenesis of Chikungunya virus and describe the factors which contribute to the processes of virus infection, spread, and disease causation.
2. Identify the role of domesticated and wild animals in the spread of Chikungunya virus.
3. Recall the epidemiology of Chikungunya including the distribution and determinants of disease.
4. Identify preventive measures and vector control procedures that can be applied to reduce the transmission of Chikungunya virus and how they may differ across populations.
5. Based on clinical signs, symptoms and relevant diagnostic tests of a patient, differentiate between Chikungunya fever and other differential diagnoses including: Dengue, typhoid, Lyme disease, rheumatoid arthritis, West Nile and systemic lupus erythematosus.
6. Recall the proper treatment protocols for a patient diagnosed with Chikungunya fever.

ABOUT THE AUTHORS

Dr. Renee Prater, DVM, MS, Ph.D., is a veterinary clinical pathologist who joined the Edward Via College of Osteopathic Medicine (VCOM) as Assistant Professor and Chair of Microbiology in 2002 after completion of her veterinary degree, pathology residency, and Ph.D. in immunotoxicology at Virginia Tech. In 2006, she was promoted to Associate Professor and appointed as Chair of Immunology at VCOM, and now serves as Associate Dean for Curriculum, Assessment, and Medical Education at VCOM. She teaches throughout the preclinical curriculum and maintains an active research program in child health and preventive medicine

Dr. Teresa Johnson, M.S. Ph.D., earned a Master of Science and a Doctor of Philosophy in Microbiology and Immunology from Virginia Commonwealth University School of Medicine and Vanderbilt University School of Medicine, respectively. She joined the National Institutes of Health Vaccine Research Center as a post-doctoral fellow in 2000 and was promoted to a Staff Scientist in 2001 where she worked on the immunopathogenesis and vaccine development for respiratory syncytial virus, continuing the vaccine development at GenVec, Incorporated as a Senior Research Scientist in 2011. Dr. Johnson joined the faculty of VCOM Virginia Campus in 2013 as Assistant Professor and Chair of Microbiology and Immunology, teaching medical students throughout the preclinical curriculum and post-baccalaureate pre-medical students. She also maintains active research programs in viral pathogenesis, development of vaccines and diagnostic tools for viral and bacterial diseases, and prevention and treatment of skin cancer.

Mrs. Alexis Stoner, MPH, Ph.D. Candidate, is currently a Clinical Instructor and Course Director of Epidemiology, Clinical Prevention and Population Health at VCOM Carolinas Campus. She has a Bachelor of Arts in Biology form Kenyon College, Masters of Public Health from The Ohio State University and is currently a Ph.D. candidate in Instructional Design and Technology at Virginia Polytechnic Institute and State University. Her research interests and practices include international environmental and women's health, health literacy among the underserved populations, and improving educational outcomes within authentic learning environments.

Dr. Matthew Cannon, D.O., is a practicing Family Medicine physician. Presently he also serves as Chairman of the Department of Family Medicine at the Edward Via College of Osteopathic Medicine. He obtained his D.O. degree from the West Virginia School of Osteopathic Medicine. He completed an Internship in Obstetrics and Gynecology at Good Samaritan Hospital in Cincinnati, Ohio and completed his residency in Family Medicine at Spartanburg Regional Medical Center in Spartanburg, S.C. He maintains his practice in primary care and also teaches as an Assistant Professor in the preclinical curriculum at VCOM. His interest in International missions has led to his research in international environments.

Dr. Nammalwar Sriranganathan, B.V.Sc, M.V.Sc., Ph.D. is a veterinary microbiologist. His Bachelors and Masters degrees in veterinary medicine and microbiology are from India. He did his doctoral in molecular biology of microbes from Oregon State University. He is a professor at Virginia Maryland College of Veterinary Medicine. His primary research focus is on vaccine development against intracellular pathogens. He does enjoy exploring nanoparticle based drug delivery, interested in understanding latency in case of intracellular pathogens and developing diagnostic tests for the detection of such latently infected animals. He teaches clinical microbiology for the senior veterinary students in their laboratory services clerkship rotation. He thoroughly enjoys mentoring graduate students in his laboratory and their progress in their careers.

BACKGROUND INFORMATION

Prior to completion of the case study, the student will be expected to pre-read this background information section, to gain an appreciation of the virology, epidemiology, and public health significance of Chikungunya virus as it applies to animal, human, and environmental health.

Emerging Diseases

The Centers for Disease Control define emerging infectious diseases as, "infectious diseases whose incidence in humans has increased in the past two decades or threatens to increase in the near future." These diseases often do not respect national boundaries and may result from changes or evolution of existing organisms, known infections spreading to new geographic areas or populations, previously unrecognized infections appearing in areas undergoing ecologic transformation, or old infections that are re-emerging due to antimicrobial resistance or breakdowns in public health measures. Severe acute respiratory syndrome (SARS), Lyme disease, Ebola in West Africa and bubonic plague are all examples of emerging diseases [1].

Our world has experienced many significant and rapid environmental changes, including urbanization, global warming, deforestation, and increased human global travel. These influences collectively and importantly alter the nature of the environment, geographic distribution of vectors, and transmission of diseases that all contribute in some way to the spread of infectious diseases.

One Health

One Health has been defined as "the collaborative effort of multiple disciplines - working locally, nationally, and globally - to attain optimal health for people, animals and the environment [2]. The delicate interaction between humans, animals and the environment is certainly not a new concept. This interaction and the balance that is required was recognized in the literature as early as the times of Hippocrates [3]. Recently, the number of newly recognized emerging infectious diseases has contributed to a re-emphasis of the One Health philosophy to better educate healthcare professionals on how to optimize management of these diseases. The diagram below illustrates an important intersection between animals, humans, and ecosystems. Importantly, it must be realized that alterations of factors in any of the three components contribute significantly to changes in disease patterns. For example, the increased frequency of severe El Nino rains creates standing water around houses, which increases reproduction and genetic alterations in the odorant receptors of mosquitoes which results in preferential feeding on humans rather than animals [4]. Another example involves the expanding human populations (numerically and geographically) which encroach on previously forested lands and contribute to increased transmission of numerous mosquito-borne diseases such as West Nile fever and dengue fever.

Figure 1. One Health. As our interaction with the environment and the ecosystems around us change, human and animal health, the ecosystem, and the environment may be dramatically affected [5]. http://www.phac-aspc.gc.ca/owoh-umus/index-eng.php All Rights Reserved. *One Health-Inter-Sectoral Approach.* Public Health Agency of Canada, 2013. Reproduced with permission from the Minister of Health, 2015. The Public Health Agency of Canada does not assume any responsibility for any errors or omissions which may result from modifications, revisions, adaptations and/or translation.

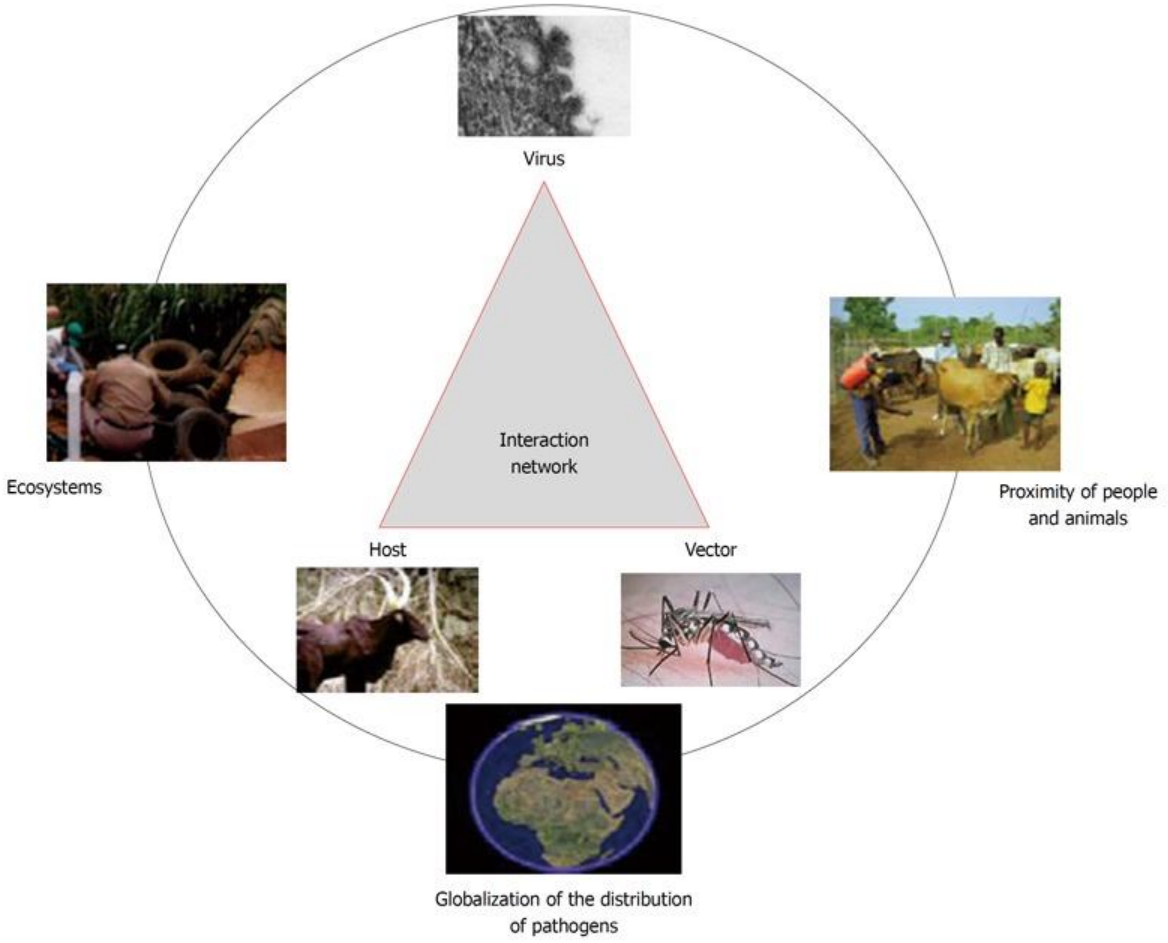

Figure 2. Globalization of the distribution of pathogens. Climate change, ecosystems evolution, anarchic urbanization, human behaviors, migration of humans and animals, development of air transport, extensive agriculture and water control projects, contribute to rapid spread of vectors and arbovirus-induced diseases in the world [6]. Available from: URL: http://www.wjgnet.com/2220-3249/full/v1/i1/11.htm

CHIKUNGUNYA VIRUS

Description of the virus

Chikungunya virus (CHIK-V) is a member of the family *Togaviridae* and the genus *Alphavirus*. As with other family members such as West Nile, yellow fever, Western equine encephalitis, and Japanese equine encephalitis viruses, CHIK-V has a single-stranded positive sense RNA genome which encodes the surface proteins E1 and E2 along with other essential proteins. These proteins are inserted in the viral envelope and are bound together, extending beyond the envelope to form the outer protein layer surrounding the virus (see diagram in Figure 3 below).

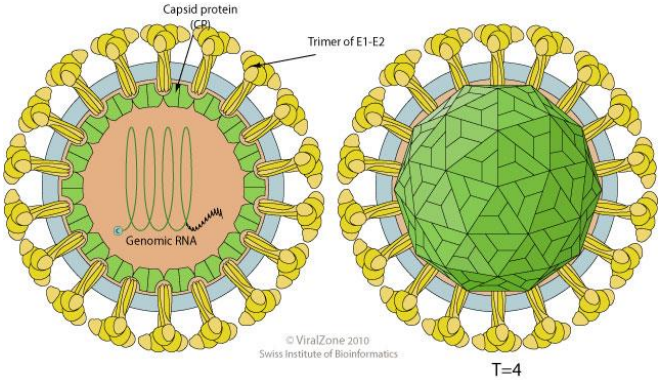

Figure 3. Togaviridae. Enveloped, spherical, icosahedral, 65-70nm in diameter, capsid with a T=4 icosahedral symmetry made of 240 monomers. The envelope contains 80 trimer spikes, each spike = 3 x E1/E2 heterodimers [7]. Image reprinted by permission from SIB Swiss Institute of Bioinformatics, ViralZone, Philippe Le Mercier.

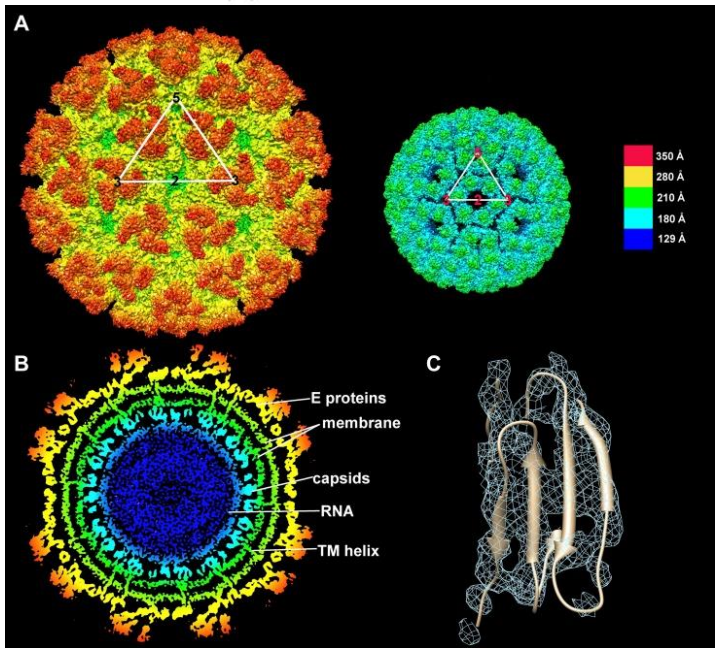

Figure 4. Structure of the CHIK VLPs. (A) Surface-shaded figure of ectodomain (left) and surface-shaded figure of nucleocapsid (right), colored according to the radial distance from the center of the virus. White triangles indicate one icosahedral asymmetric unit. (B) Cross-section of the virus showing density above 1.5 σ also colored according to the radial distance from the center of the virus. (C) Resolution of β-strands in the E1 domain III [8].

These two proteins, E1 and E2, serve as viral fusion and attachment proteins (respectively), adhering to receptors (likely prohibitin) on host cells [9]. Binding of the E1/E2 complex to the host cell receptor triggers a conformational change, exposing the fusion domain on the E1 protein, allowing fusion of the viral and host cell membranes as shown in Figure 5 below. The virus particle is then internalized by endocytosis through clathrin-coated pits. The CHIK-V E1 glycoprotein mediates fusion of the virus particle to the host cell plasma membrane and therefore plays a major role in the pathogenic process of the virus. Mutations in this protein have been shown to have significant impact on the virus replication cycle (see discussion below). Also, based on the clinical success of HIV-1 fusion inhibitors, this CHIK-V protein may be a potential target for similar anti-viral drug development.

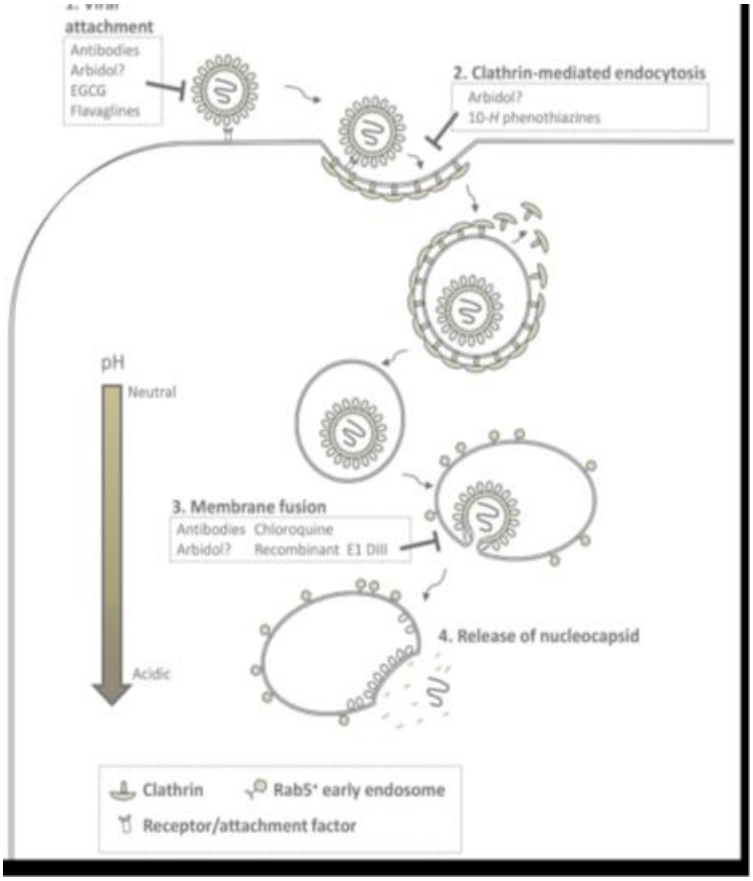

Figure 5. Chikungunya virus cell entry and potential antiviral strategies. The viral life cycle starts with attachment of the virus particle to one of the ubiquitously expressed attachment factors or receptors at the cell surface (**1**). Subsequently, the virus is internalized into the cell via clathrin-mediated endocytosis (**2**). Then, clathrin-molecules dissociate from the vesicle and the virus is delivered to Rab5+ endosomes. Within the mildly acidic lumen of the endosome, the viral glycoproteins E2 and E1 undergo major conformational changes that lead to membrane fusion (**3**); Thereafter, the nucleocapsid core is released into the cytosol (**4**). The molecules and compounds that are known to interfere with entry are stated in the boxes [10]. Information that is created by or for the US government on this site is within the public domain. Public domain information on the National Library of Medicine (NLM) Web pages may be freely distributed and copied.

Initial infection occurs in the dermal fibroblasts at the site of infection, followed by viral replication and dissemination with infection of skeletal muscles, myotendinous insertions, and joint capsules. Additionally, the CHIK-V E1 and E2 proteins, targets of virus-specific antibody production, are the basis for antibody-dependent enhancement (ADE) of infection: antibodies bind the viral particles, and then the antibody-virus complex is bound by Fc receptors of monocytes and macrophages, which facilitates infection of these cells (see Figure 6 below). These infected monocytes and macrophages freely migrate throughout the body which allows dissemination of the virus to multiple target organs. The transportation of virus in these virus-infected macrophages then allows disease manifestations of fever, back pain, headache, myositis, polyarthralgia, and, in some cases, rash (see discussion below).

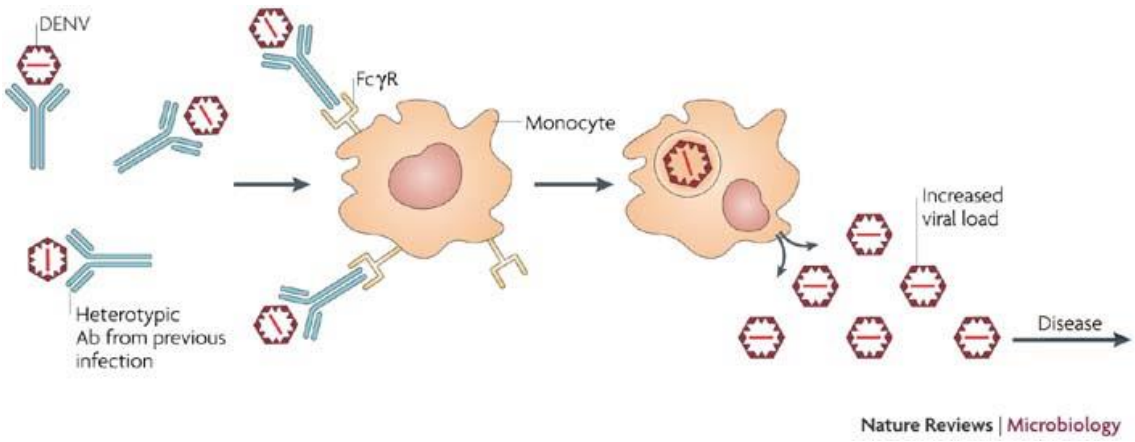

Figure 6. Antibody (Ab)-dependent enhancement of virus replication occurs when heterotypic, non-neutralizing Ab present in the host from a primary dengue virus (DENV) infection binds to an infecting DENV particle during a subsequent heterotypic infection but cannot neutralize the virus. Instead, the Ab–virus complex attaches to the Fcγ receptors (FcγR) on circulating monocytes, thereby facilitating the infection of FcγR cell types in the body not readily infected in the absence of antibody. The overall outcome is an increase in the overall replication of virus, leading to the potential for more severe disease [11]. Image reproduced with permission from Macmillan Publishers Ltd.

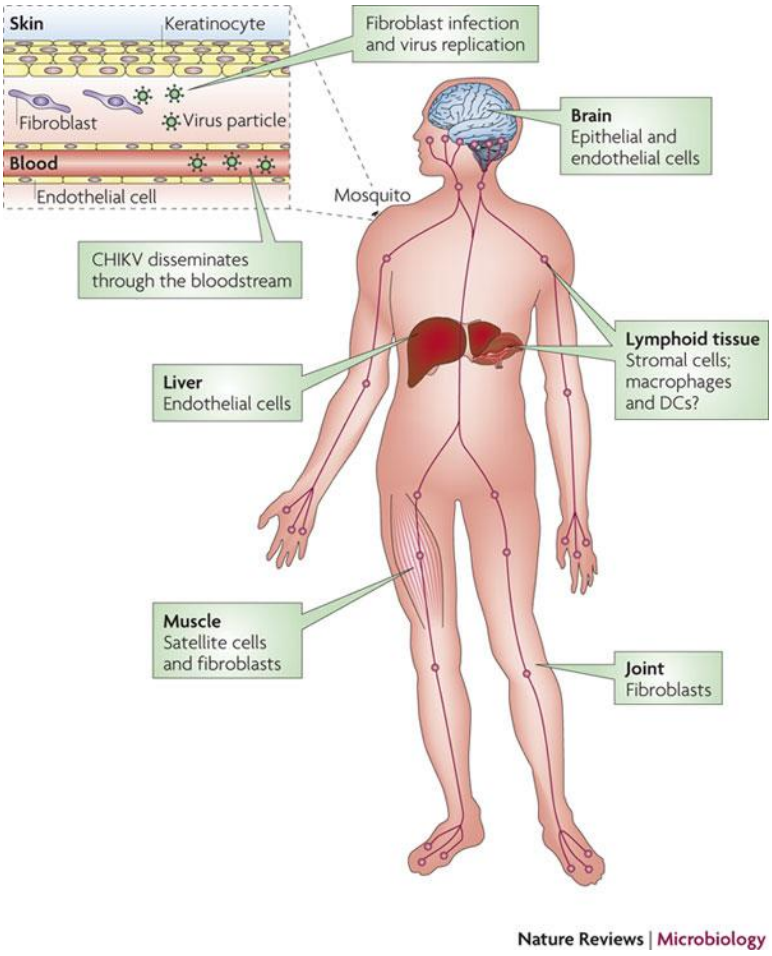

Figure 7. Transmission of Chikungunya virus (CHIK-V) occurs following a mosquito (*Aedes aegypti* or *Aedes albopictus*) bite. CHIK-V then replicates in the skin, in fibroblasts, and disseminates to the liver, muscle, joints, lymphoid tissue (lymph nodes and spleen) and brain. The target cells are indicated for each tissue [12]. Image reproduced with permission from Macmillan Publishers Ltd.

Transmission of Vector-Borne Diseases such as CHIK-V

Many diseases, including CHIK-V, are transmitted in a blood-borne fashion from one individual to another through the bite of mosquitoes. Mosquitoes have a rather complex life-cycle, depicted in Figure 8 below, in which the female mosquito lays her eggs in an aquatic environment; the immature forms remain aquatic whereas the adult form is terrestrial.

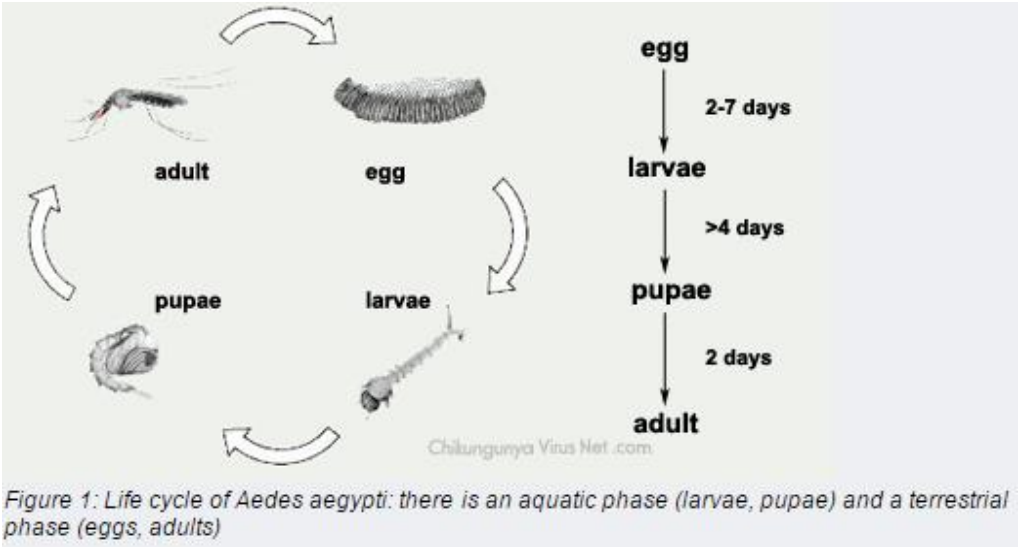

Figure 1: Life cycle of Aedes aegypti: there is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults)

Figure 8. Life cycle of *Aedes aegypti*: there is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults) [13]. Image reproduced with permission from Centers for Disease Control.

While both males and females may receive nourishment from nectar and plant fluids, only the female bites mammals, in order to use the blood meal as a protein source for egg development. When the female bites (typically in the mornings and evenings, although feeding can occur throughout the day), she injects a small amount of saliva to delay the host blood clotting process. Pathogens such as CHIK-V which replicate in the salivary glands of the mosquito may be shared with new hosts during this injection. Only about 2 microliters of blood are withdrawn by the female mosquito during each blood meal, requiring her to feed on multiple hosts to consume the blood volume required to complete development of her eggs. Additionally, since the virus incubation is 8-10 days, and the female mosquito typically lives 2-3 weeks, the female mosquito is capable of transmitting the virus to a number of individuals during her lifetime. There is also evidence that the virus may be transmitted trans-ovarially (via the eggs), although the ability of the offspring to then infect another animal or person is not clearly understood.

There are two critical factors required for the transmission cycle to be completed. First, is the ability of the virus in the ingested blood to infect cells of the midgut of the mosquito then disseminate to and replicate in the salivary glands (see Figure 9 below). Second, the virus must replicate in the local dermal fibroblasts and migrating macrophages of infected animals to reach the vascular endothelium to replicate and establish viremia at a concentration sufficiently high that the virus may be transmitted back to a mosquito during feeding. During acute CHIK-V viremia, at the peak of transmissibility viral titers typically range from10^9 to 10^{12} particles per milliliter of blood [12]. As the virus replicates in the infected host, it is disseminated to tissues as the infected macrophages migrate throughout the body, potentially establishing persistent infection. Infected macrophages may also traffic to the joint spaces where local virus-specific immune responses and/or an autoimmune response create an inflammatory environment, leading to the arthralgia seen in acute and recurrent disease as shown in the diagrams below [14, 15].

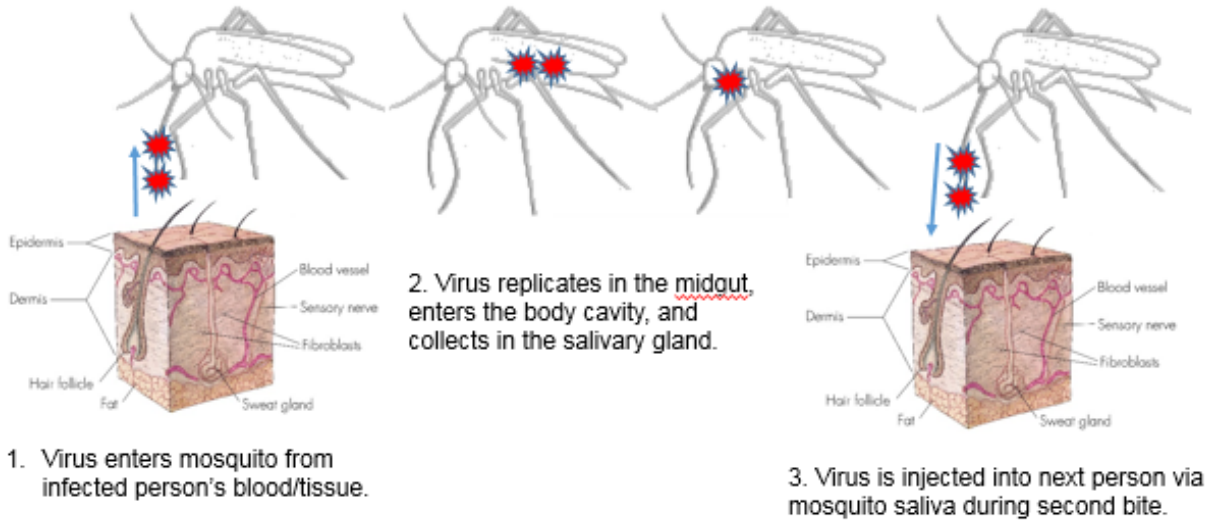

Figure 9. Process of infection and transmission of CHIK-V by female mosquito vectors. The mosquitoes that carry CHIK-V ingest the virus from infected host blood and tissues during bloodfeeding. The virus enters the midgut and then enters the body cavity. The virus then infects mosquito salivary glands, and is secreted into saliva during the next blood meal. Since female mosquitoes often require multiple small blood meals to complete development of her eggs, she is likely to transmit CHIK-V from one host to another in saliva.

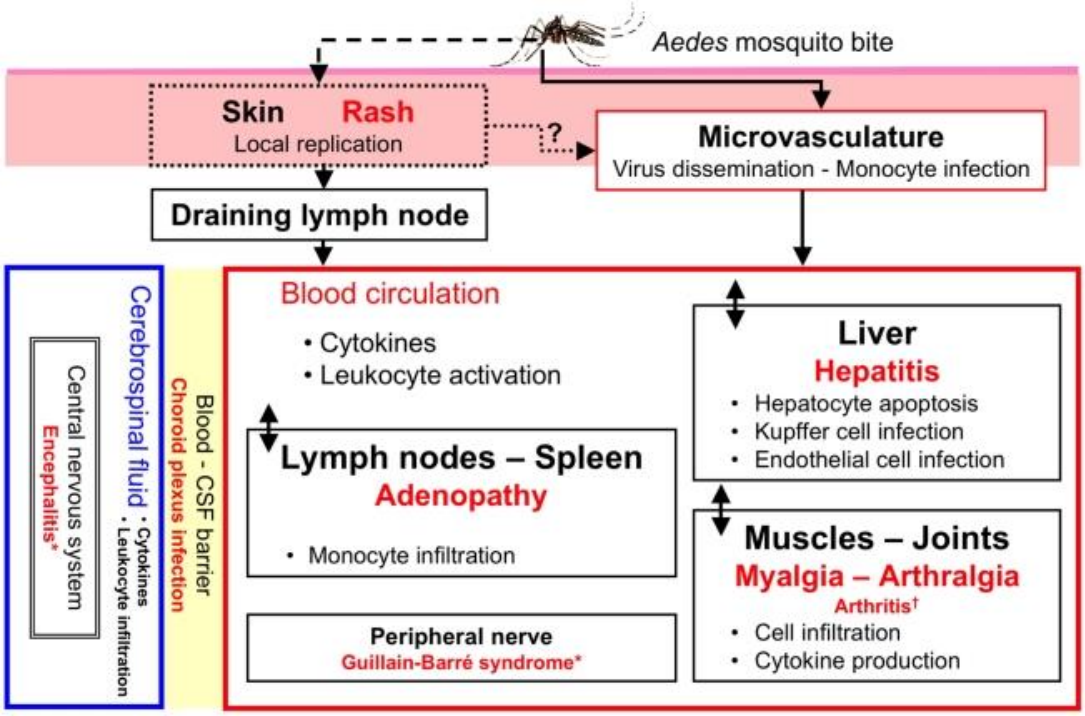

Figure 10. Virus dissemination and target organs. Following inoculation with CHIKV through a mosquito bite, the virus directly enters the subcutaneous capillaries, with some viruses infecting susceptible cells in the skin, such as macrophages or fibroblasts and endothelial cells. Local viral replication seems to be minor and limited in time, with the locally produced virus probably being transported to secondary lymphoid organs close to the site of inoculation. The blood carries most

viruses, as free virions or in the form of infected monocytes, to the target organs, the liver, muscle, joints, and remote lymphoid organs. In these tissues, infection is associated with a marked infiltration of mononuclear cells, including macrophages, particularly when viral replication occurs. The pathological events associated with tissue infection are mostly subclinical in the liver (hepatocyte apoptosis) and lymphoid organs (adenopathy), whereas mononuclear cell infiltration and viral replication in the muscles and joints are associated with very strong pain, with some of the patients presenting arthritis. * Guillain-Barré syndrome and encephalitis are very rare events. † True arthritis remains a rare event (from 2% to 10%) [14]. Image was obtained from an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

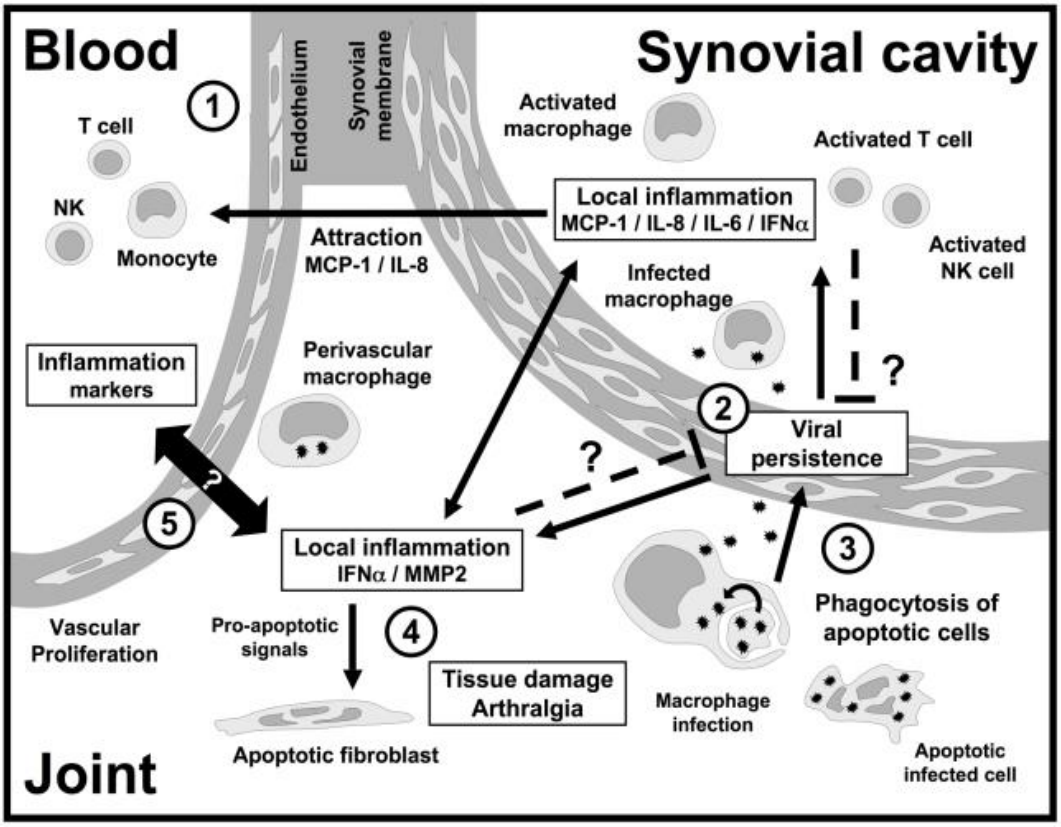

Figure 11. Mechanisms of CHIKV persistence and tissue inflammation in patients with chronic disease. (1) Months after the acute infection, monocytes, T cells, and natural killer (NK) cells are still attracted to the inflamed joint, where they become activated. (2) The infection of macrophages in joints is associated with local inflammation and the production of cytokines, chemokines, and pro-inflammatory effectors, such as MCP-1/CCL-2, IL-8, IL-6, IFN-α, and MMP2. (3) The phagocytosis of apoptotic bodies from infected cells probably contributes to viral persistence. Nevertheless, the beneficial or deleterious effect of local inflammation on viral persistence remains unclear. (4) When it occurs, arthritis is accompanied by high rates of fibroblast apoptosis and cartilage destruction. Chronic inflammation probably plays a major role in this damage and associated pain. (5) The potential relationship between local inflammation of the joint and a state of systemic activation, as demonstrated by the presence of inflammation markers in plasma and blood cells, remains unclear [14]. Image was obtained from an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

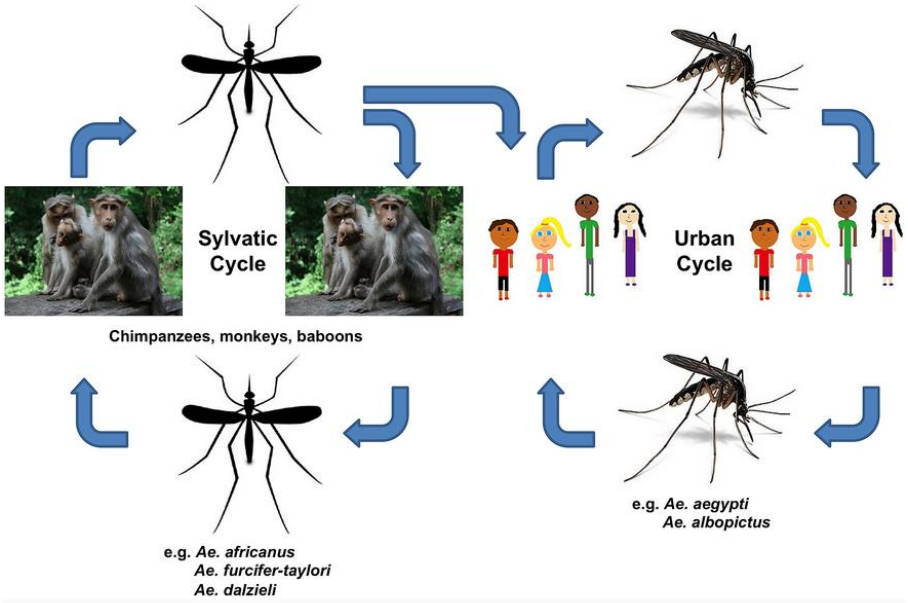

Figure 12. Life cycle of Chikungunya virus in Africa showing the interconnection between the sylvatic cycle on the left and the urban cycle on the right. Particularly in Africa, the virus is maintained in a sylvatic cycle comprising non-human primates and different species of forest-dwelling mosquitoes including *Aedene* mosquitoes (*Ae. africanus*, *Ae. furcifer-taylori*, *Ae. dalzieli*, etc.) and non *Aedene* mosquitoes (Mansonia, Culex, etc.) [16]. Image was obtained from an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Evolution of the virus and adaptation to the vector

Documented re-emergence of CHIK-V into the human population occurred in the early 1950s in sub-Saharan Africa. This viral strain then spread - first to Central/East Africa and then to numerous Southeast Asian countries surrounding the Indian Ocean. Genetic drift, including minor mutations in the CHIK-V E1 and E2 viral attachment/fusion proteins, occurred and gave rise to three distinct but related lineages - the West African, the East/Central/South African (or ECSA), and the Asian genotypes. However, these genetic changes did not alter the infectivity nor tropism of these three genotypes. These viruses replicate in various sylvatic *Aedes* species (e.g. *Ae. africanus*, *Ae. taylori*, *Ae. furcifer*) which populate the deep forest and jungle areas in Central and West Africa. In contrast, *Ae. aegypti*, which has a more urban life cycle with closer human contact, appears to be the dominant vector in Asia. In 2004, a single point mutation appeared in the ECSA lineage during an outbreak in coastal Kenya. This mutation, designated A226V, converted the alanine codon at position 226 of the E1 fusion protein to a valine codon. This mutation has been shown to enhance the fusion activity of the E1 glycoprotein and increase virion stability [17], markedly altering the CHIK-V transmission cycle in two ways. First, by facilitating virus fusion, this mutation allows mosquitoes to be infected with a lower inoculum of virus during feeding which has enabled *Ae. albopictus* to become a vector for CHIK-V. Also, the stabilized virus particle and enhanced fusion activity of the E1 A226V virus strain results in increased viremic titers in both the host and the vector which, in turn, increases virus transmission between humans and mosquitoes. This virus strain rapidly spread throughout the Indian Ocean region and Southeast Asia, causing the strain to be designated the Indian Ocean Lineage (IOL). Transmission and spread of the IOL strain was enhanced since this mutation resulted in adaptation of the virus to *Ae. albopictus* (commonly called the Asian tiger mosquito) as a vector. Due to variations in the

territories and feeding habits between *Ae. aegypti* and *Ae. albopictus* (see "Transmission Pathways" below), transmission to humans has been dramatically enhanced with increased frequency of infection and broader geographic distribution of infections.

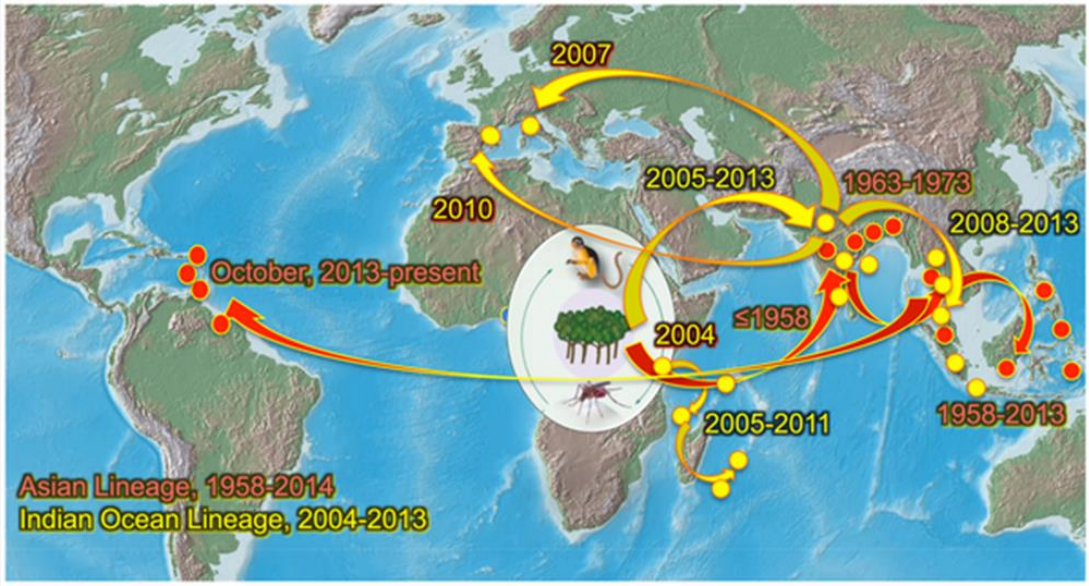

Figure 13. Map showing the distribution of Chikungunya virus enzootic strains in Africa and the emergence and spread of the Asian lineage (red arrows and dots) and the Indian Ocean lineage (yellow arrows and dots) from Africa [18]. Image was obtained from an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

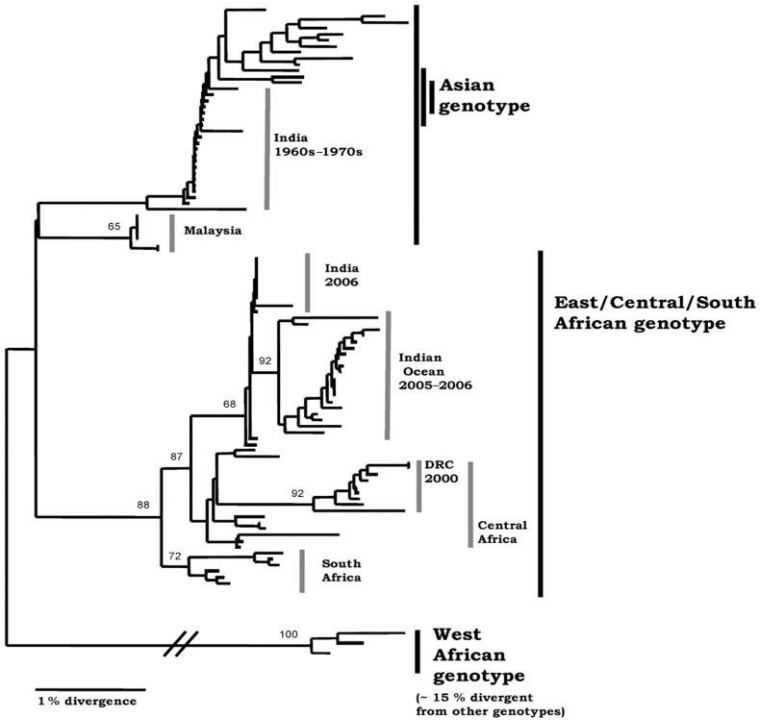

Figure 14. Phylogram of 99 CHIK-V E1 sequences demonstrating the main genotypes and close relationships among the lineages from each genotype based upon geography and time of the outbreak. Numbers at nodes indicate bootstrap support of 1000 replications [19]. Image was obtained from an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Carriers of Chikungunya Virus

In Africa, India, and Southeast Asia, a sylvatic transmission cycle is maintained between infected non-human primates and *Aedes spp*. mosquitoes (see Figure 12 above). In China, bats have also been found to be infected with CHIK-V. To date, existence of a rural transmission cycle with infection of livestock, dogs, or cats has not been demonstrated. However, asymptomatic infection of rodents has been documented. Animal reservoirs have yet to be identified in the newly infected geographies of the Western Hemisphere, suggesting mosquito-human-mosquito is the primary mode of infection.

With development of an urban transmission cycle for CHIK-V, a concern within the medical community has been the possibility for livestock and domesticated animals to be infected and serve as reservoirs for CHIK-V, increasing transmission to human populations living in close contact with these animals. Some studies have shown that while livestock (e.g. cattle) may produce CHIK-V antibodies, the titers tend to be at the limit of assay detection [20]. Additionally, analyses of sera from domesticated and wild mammals and poultry present in the midst of a major epidemic demonstrate that, except for non-human primates, these animals rarely have antibodies against CHIK-V (see Figure 15), and in these animals replicating virus is only detectable in non-human primates [21]. These data are seen to support the hypothesis that, while domesticated and wild animals may be exposed to CHIK-V, most (except for non-human primates and bats) cannot support virus replication at all or at a level that produces viral titers in the blood high enough for mosquitoes to be infected during feeding.

While it currently appears that domesticated animals do not play a direct role in the transmission of CHIK-V due to very low levels of viremia, these animals can indirectly contribute to the spread of disease in several ways. While livestock and pets may not support sufficient CHIK-V replication to transmit virus to mosquitoes, they serve as sources of blood meals and, thereby, increase the presence of mosquitoes in the human environment. Livestock also enhance the incidence of mosquitoes around human dwellings due to the need for water containers for the animals as the standing water may frequently serve as a breeding ground for CHIK-V-infected mosquitoes. Thus, while domesticated animals currently seem to manifest sub-clinical disease and have low probability of transmitting CHIK-V to mosquitoes, as CHIK-V continues to spread into new environments and human populations, this may change. The ability of minor genetic changes to have dramatic impact on CHIK-V replication and vector tropism has been shown with the emergence of the A226V point mutation and the IOL isolate.

With escalating interactions of CHIK-V with our domesticated animals, the virus will likely continue to evolve and adapt to these hosts, possibly increasing CHIK-V virulence in domesticated animals. Therefore, the veterinary and medical communities would be advised to monitor livestock, pets, and rodent populations in human communities for the appearance of clinically apparent disease caused by CHIK-V variants which may produce sufficiently high viral titers in the blood of the domesticated animals to allow transmission to the feeding mosquito and then to humans. This is an area where vigilance and mosquito control may help us in minimizing or preventing the development and spread of such genetic variants of CHIK-V.

Table 1 Number of specimens tested by qRT-PCR and by ELISA, and number of seropositive animals

		qRT-PCR	ELISA	
Species	Sampling location	Number tested*	Number tested	Number positive (%)
Domestic carnivores				
Cat - *Felis catus*	Reunion	38	37	0
Dog - *Canis lupus*	Reunion	69	68	0
Farm mammals and poultry (Reunion Island)				
Horse - *Equus ferus*	Reunion	76	97	0
Cattle - *Bos primigenius*	Reunion	115	116	0
Goat - *Capra aegagrus*	Reunion	95	115	0
Sheep - *Ovis aries*	Reunion	25	49	0
Pig - *Sus scrofa*	Reunion	48	108	0
Poultry - *Gallus gallus*	Reunion	37	113	0
Wild mammals				
Shrew - *Suncus murinus*	Reunion	105	45	0
Ship rat - *Rattus rattus*	Reunion	74	75	3 (40%)
Norway rat - *Rattus norvegicus*	Reunion	6	17	0
House mouse - *Mus musculus*	Reunion	33	20	0
Reptiles				
Panther chameleon - *Chamaeleo pardalis*	Reunion	17	not tested	not tested
Non-human primates				
Brown lemur - *Eulemur fulvus*	Mayotte	53	52	2 (3.8%)
Crab-eating macaques - *Macaca fascicularis*	Mauritius	not tested	134	1 (0.7%)
Crab-eating macaques - *Macaca fascicularis*	Reunion	not tested	1	1 (100.0%)
Hamadryas Baboon - *Papio hamadryas*	Reunion	not tested	2	0
Southern Pig-tailed Macaque - *Macaca nemestrina*	Reunion	not tested	1	0
Campbell's Monkey - *Cercopithecus campbelli*	Reunion	not tested	1	0
Total		**791**	**1051**	**7**

*All samples were negative by qRT-PCR

Figure 15. Number of specimens tested by qRT-PCR and by ELISA, and number of seropositive animals [21]. Image was obtained from an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Transmission Pathways

With the historic African, ECSA, and Asian CHIK-V genotypes, transmission occurs between infected non-human primates or bats (Asia only) with the *Ae. aegypti* mosquito serving as the arthropod vector for human infection. The newly emerged IOL CHIK-V genotype containing the A226V E1 point mutation may be transmitted from infected animals to humans by both *Ae. aegypti* and *Ae. albopictus* mosquitoes. This newly emergent pathway of transmission has already had dramatic impact on the incidence of human disease. It is believed in the medical and scientific community that this newly acquired ability of CHIK-V to infect and be transmitted by the *Ae. albopictus* mosquito will greatly accelerate the global spread of the virus to previously CHIK-V-naive locales and populations. Four major distinctions between *Ae. aegypti* and *Ae. albopictus* are considered to be primary factors in the potential for enhanced CHIK-V transmission (see following 4 points):

- *Geographic distribution of Ae. aegypti and Ae. albopictus* - While originally restricted to Southeast Asia, the *Ae. albopictus* mosquito has been introduced into the Western Hemisphere and Europe over the past ~25 years due to increased industrial commerce with importation of tires from China playing a major role due to the presence of standing water and *Ae. albopictus* mosquito larva inside the tires.

- *Feeding behaviors* - While the *Ae. aegypti* mosquito does feed during daylight hours (particularly when cloudy), this is a limited behavior as the mosquito predominantly feeds at dawn and dusk. In contrast, *Ae. albopictus* feeds with equal frequency throughout the

daylight hours, from dawn to dusk. Furthermore, the *Ae. albopictus* mosquito is markedly more aggressive in its feeding behaviors.

- *Habitat - Ae. aegypti* generally inhabits the areas surrounding human habitats, preferring standing and stagnant water in containers (e.g. vases, pots, discarded tires, toilet tanks, open drains such as in showers). This mosquito does not typically enter the deep forest. *Ae. albopictus*, however, freely roams between established human habitats and heavily wooded areas. Thus, the habitat of *Ae. albopictus* includes both human and enzootic hosts which may facilitate CHIK-V transmission, particularly as local communities enact insect control measures.

- *Temperature tolerance - Ae. aegypti* flourishes in the warm tropical climates of Africa, Southeast Asia, and Central America (see areas between red lines in Figure 16 below), but the larvae are killed by the winter temperatures of more temperate climates. This is not the case for *Ae. albopictus* whose larvae can survive during the winter in these colder, but temperate, climates. This provides for a significantly greater geographic distribution of the *Ae. albopictus* mosquito (see gold highlights in Figure 16 below) and allows for the potential infection of new human populations with no prior antigenic exposure to CHIK-V.

Comparison of the geographic distribution of *Ae. aegypti* (between red lines) and *Ae. albopictus* (gold fill) domains can be found in the map (Figure 16) below, which clearly demonstrates that *Ae. albopictus* mosquitoes cover a wider geographic area. This information suggests that a wider worldwide distribution of the insect vector will result in spread of the CHIK-V disease beyond the traditionally tropical areas of disease incidence.

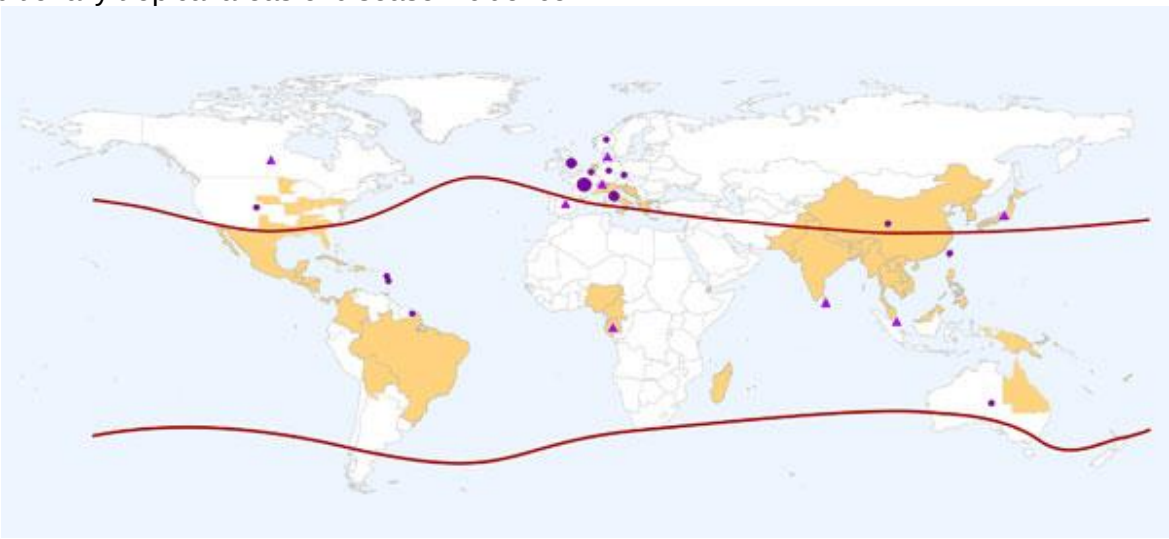

Figure 16. Imported cases of Chikungunya virus infection and known and theoretical geographic distributions of *Aedes albopictus* and *Ae. aegypti* mosquitoes. World repartition of *Ae. albopictus* mosquitoes (gold areas) and theoretical dispersion of *Ae. aegypti* in 2008 (the band between red lines, which represent the 10°C isotherms) according to the World Health Organization. Areas where imported cases of Chikungunya have been reported during 2005–2008 are marked with a purple circle (small: 1–73 cases; medium: 74–300 cases; large: >300 cases) or a purple triangle when the number of imported cases was unknown. Data sources: US Centers for Disease Control and Prevention, World Health Organization, and literature review on Medline by Pubmed. Map drawn using ARCGIS version 9.2 (www.esri.com/software/arcgis) [22]. Image reproduced with permission by Emerging Infectious Diseases Journal.

EPIDEMIOLOGY OF CHIK-V

The global and US disease distribution of CHIK-V are pictured in Figures 17 and 18 below:

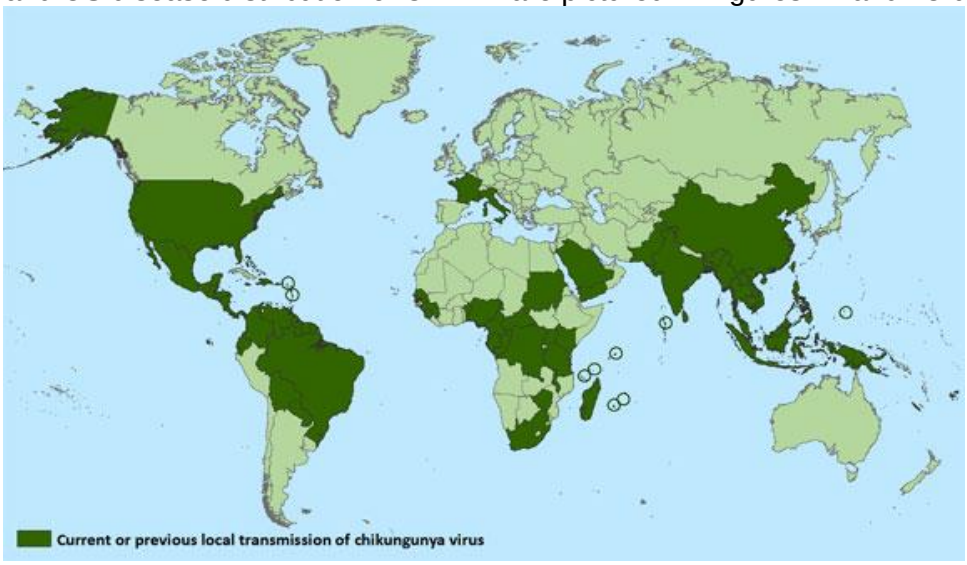

Figure 17. Countries and territories where Chikungunya cases have been reported* (as of March 10, 2015) [23]. Chikungunya virus has been reported in many countries and territories all over the world; although the origins are believed to be Asia and Africa, factors such as increased global travel, and spread of mosquito vectors, have resulted in an expanding global distribution of the disease. The worldwide distribution depicted in the graph above is current *as of March 10, 2015.* Image reproduced with permission from the Centers for Disease Control.

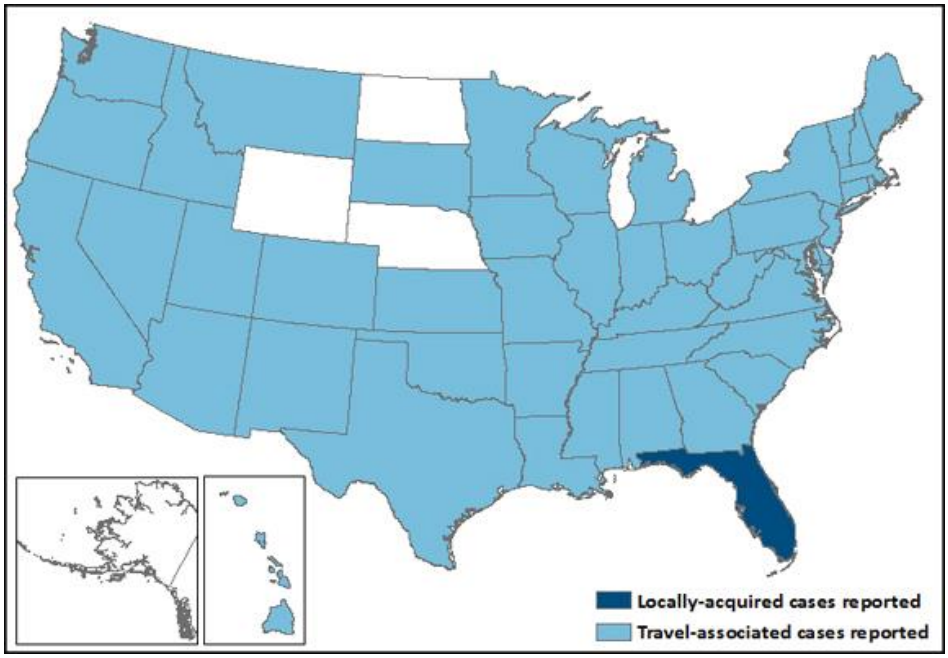

Figure 18. States reporting Chikungunya virus disease cases – United States, 2014 [24]. Image reproduced with permission from the Centers for Disease Control.

Although historically a disease of the Far East and sub-Saharan Africa, Chikungunya virus and its mosquito vectors have recently spread to Central America and now into the United States. The map above, using 2014 data, demonstrates that the disease has been reported in nearly every state in the U.S., although at that time, the cases outside of Florida were travel-associated (traveler acquiring the virus outside the US, manifesting symptoms upon their return) rather than locally-acquired. Because of the northern progression of the two main mosquito vectors, we are now beginning to see more locally-acquired cases in the southern states (i.e. Florida). While CHIK-V was historically endemic in portions of Africa, Asia, Europe and the Indian and Pacific Islands, it was identified for the first time in the Americas on islands in the Caribbean in 2013. Cases of CHIK-V have now begun to spread into the North American continent with cases reported in 44 countries or territories throughout the Americas and with 1.2 million cases reported to the Pan American Health Organization. As of July 21, 2015, there have been a total of 237 CHIK-V disease cases from 34 US states, mostly among travelers returning from affected areas and from Puerto Rico and the US Virgin Islands, but with 11 confirmed cases in Florida caused by the bite of local, infected mosquitoes [25].

We can utilize this information of the geographic location of the mosquito vectors at certain time points (see map below) to plan judicious use of insecticides in at-risk areas. The basis of control of the disease is environmental and vector control, in the absence of effective antivirals or vaccines.

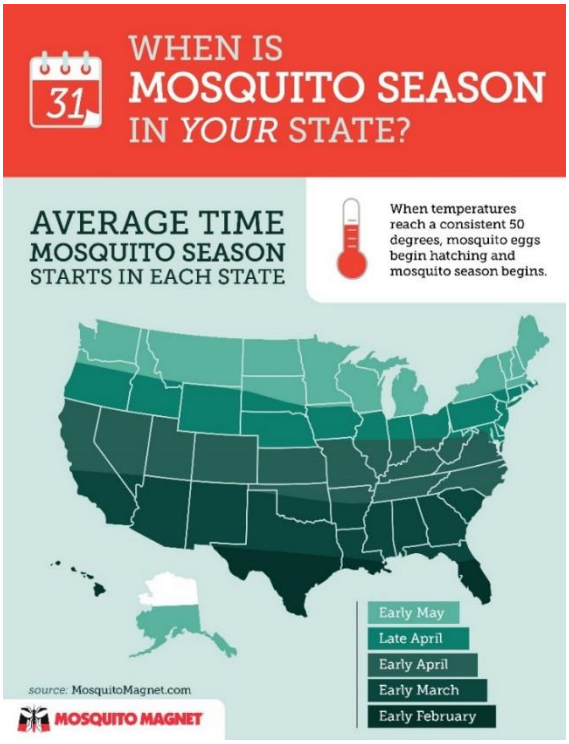

Figure 19. Mosquito season varies from year to year depending on temperature, moisture and other conditions. The map shows approximate periods when mosquitoes may be most active, with activity tapering off for a month or more before and after. Alaska's short but strong mosquito season peaks in June and July. In Hawaii, mosquitoes are least noticeable in fall, but are active most of the rest of the year [26]. Image was obtained from an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

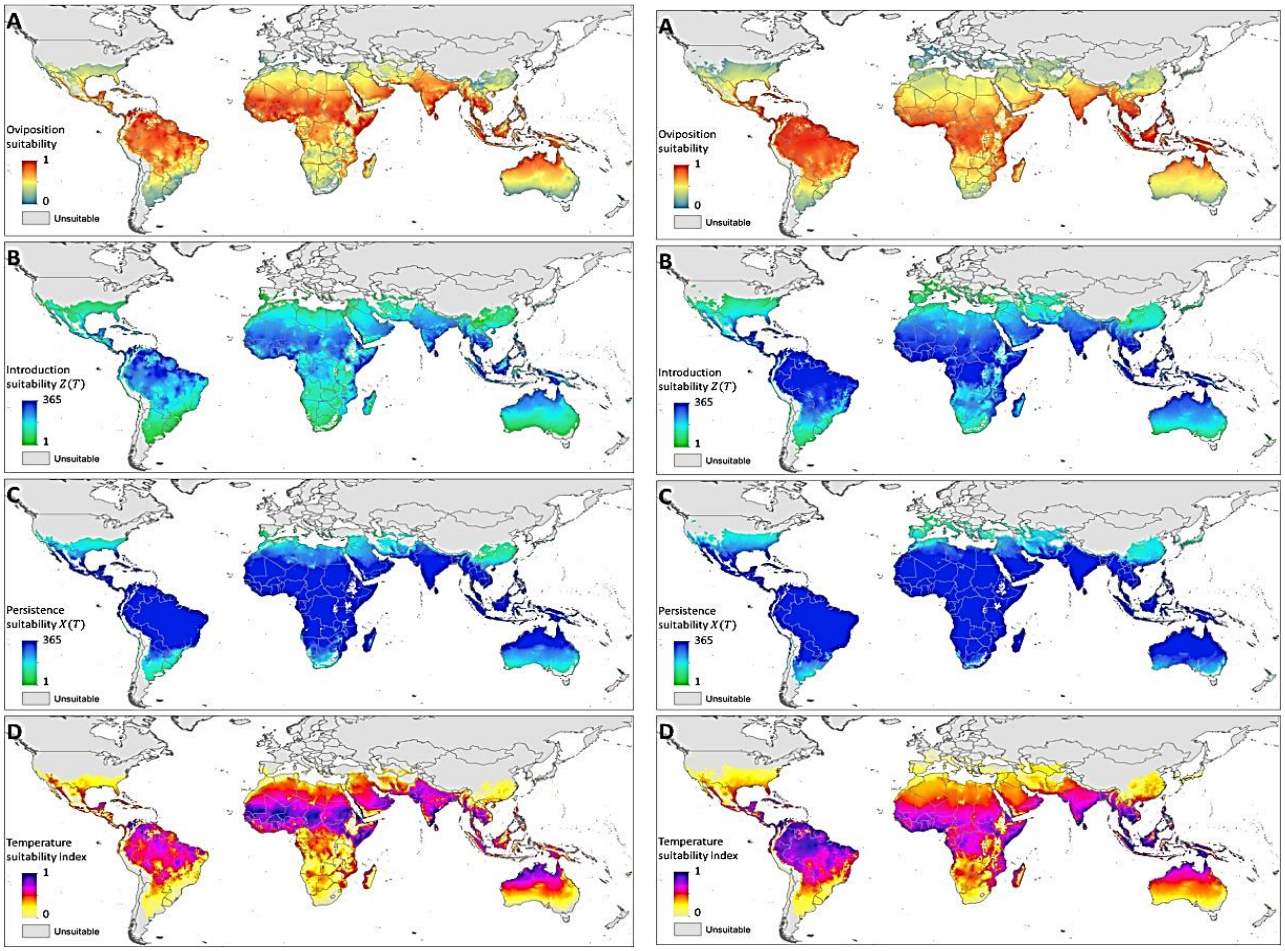

Figure 20. Left panel: *Ae. aegypti* temperature suitability for persistence and DENV transmission. (A) The annualised summary of temperature suitability for oviposition ($X_{ovi}(T)$) on a normalised scale. **(B)** Introduction suitability; the number of days in a year where introduction of a DENV infected human would lead to ongoing transmission ($Z(T)_i > 0$). **(C)** Persistence suitability; the number of days in the year where onward DENV transmission could occur if a constant source of infectious humans were available ($X(T)_i > 0$). **(D)** The annualised summary of temperature suitability ($X(T)$) on a normalised scale. Predictions in all above maps are constrained to areas that permit oviposition ($X_{ovi}(T)_i > 0$) on 219 or more days in the year, as determined by comparison with known occurrences of *Ae. aegypti*. **Right panel: *Ae. albopictus* temperature suitability for persistence and DENV transmission.** Panels correspond directly to those described for *Ae. aegypti*, but constraints are expanded to areas that permit oviposition for 365 days in the year [27].

CASE STUDIES

Please read the following case descriptions and view the two embedded videos below that compare and contrast the approach to diagnosis and management of Chikungunya virus in a first world country as compared to a third world, endemic community in Central America. This information will be helpful as the student proceeds through the remainder of the case study section, which examines the diagnosis, treatment, and control of Chikungunya virus, and highlights the role of wild and domestic animals as well as the environment in the spread and control of this emerging infectious disease. Careful consideration of the guided questions integrated throughout the case study section will help the student gain a strong appreciation of the diagnosis and management of this emerging infectious disease. For consideration as you think about patient diagnosis: As shown in Figures 16 and 20 above, both Chikungunya virus and Dengue virus are transmitted by mosquitoes. Arthropods such as mosquitoes and ticks may serve as vectors for multiple infectious agents. An interactive map may be found at http://diseasemaps.usgs.gov/mapviewer/ [44]. This link provides up-to-date disease incidence rates for eight arthropod-borne viruses in both human and veterinary populations as well as birds, mosquitoes, and sentinel animals from 2003 through 2015.

Video #1: CHIKUNGUNYA VIRUS IN A PATIENT FROM THE U.S.

A 24 year-old Caucasian female presents to a primary care office. She describes 5-day history of fever, myalgia, arthralgia and a flushed appearance involving her face and thorax. She states that the initial symptom was a fever of 101.4 degrees Fahrenheit that began this past Monday evening, 5 days ago. She also states a subsequent malaise feeling that has progressively developed. Her symptoms of arthralgia initially began in her hands and feet and have progressively involved her wrists, elbows and ankles. The myalgia involving both her upper and lower extremities has progressively increased. She states rest and continuous ibuprofen have helped minimally and any form of strenuous exertion has exacerbated her symptoms. She describes no previous history of this type of illness. She states no changes in her diet or other daily activities. Her joint pain is an 8 on a 1 to 10 scale. She was initially seen at an Immediate Care evening clinic on Wednesday evening, 2 days after the onset of symptoms in which a physical exam was performed and basic laboratory data was collected via venipuncture. She was told she likely had an infectious or non-infectious rheumatologic illness, and the differential diagnosis list included: leptospirosis, rickettsial disease, streptococcal disease, parvovirus, enterovirus, or other non-infectious rheumatological disease. Based on the nonspecific nature of her symptoms, was sent home with instructions to continue symptomatic care.

She continued to worsen, developing a maximum temperature of 104 degrees Fahrenheit on the fourth day of her illness. She returned to the same Immediate Care facility that she was treated at previously and had a more extensive set of labs performed. She was ultimately diagnosed with a cellulitis and treated with antibiotics.

Ultimately a day later she presented to a different medical provider who was able to access the patient's information and expand upon the medical work-up that had been performed. This physician obtained a comprehensive history. During this dialogue, she denied recent travel on any vacations. When she was asked, however, if she had traveled out of the country for any reason, she responded that she had recently been on a mission trip to help construct houses in El Salvador. This valuable piece of information helped lead the physician to consider diagnoses that are currently more prevalent in Central America and less prevalent in the US. Based on this patient's history as well as clinical signs and symptoms, a differential diagnosis list was expanded

to include endemic illnesses to El Salvador including malaria, acute Dengue fever typhoid fever, West Nile virus, and Chikungunya virus. In order to determine a definitive diagnosis, the physician was able to obtain a blood smear, blood culture, serologic antibody studies, Giemsa stain, ELISA studies and agglutination studies. Based upon the results of these labs, the proper diagnosis was obtained and appropriate treatment plan was implemented.

[redacted]

Video #2: CHIKUNGUNYA VIRUS IN A PATIENT FROM EL SALVADOR

During a mission trip in El Salvador, a 24 year-old female presented with a fever of 101 degrees Fahrenheit, myalgia, arthralgia, and a persistent headache. She described feeling confident that she was bitten by something, likely a mosquito. Her physical examination demonstrates tenderness to palpation in multiple muscles and joints. Based on this patient's presentation, history, and physical exam, the medical student and physician were able to conclude the likely diagnosis of her condition due to having a sound knowledge of the prevalence of some infectious diseases in that area such as acute Dengue fever, yellow fever, measles, rubella, typhoid fever, and Chikungunya virus. Because of the student's familiarity with these characteristic signs and symptoms, pathogenesis, and mode of transmission of diseases that are endemic to this region, the medical student and physician reach the proper diagnosis of Chikungunya virus in the absence of extensive or highly technical laboratory testing, or unnecessary use of medication.

[redacted]

After reading the two case presentations and viewing the respective videos, the student should appreciate three main points:

1. *While treatment of this illness is identical in the U.S. as it is in El Salvador, the approach to diagnosis may be very different in an endemic vs. a non-endemic area.*
2. *The differential diagnosis list is broader in a non-endemic area vs. an endemic area, and additional diagnostics are often helpful in narrowing that differential diagnosis list in a non-endemic area.*
3. *These case scenarios also should help the student to appreciate the importance of a strong understanding and awareness of this emerging infectious disease as it relates to prevalence, key clinical signs, and the role of animals and the environment in the spread and control of the disease.*

Questions for Students on One Health, Emerging Disease, and Epidemiology of CHIK-V

- How is Chikungunya a disease that is illustrative of the One Health Philosophy?
- Can you think of another example of an emerging disease either within the U.S. or a different country?
- Based on the One Health relationship, what factors contributed to the development and spread of disease in the example you described above?
- Why are vector-borne diseases a major concern in the U.S. and on a global scale?
- What characteristics of vector-borne disease make them difficult to control and prevent rapid transmission?
- What changes in human behavior may contribute to the spread of CHIK-V?
- What changes in human behavior may contribute to the spread of mosquito vectors for CHIK-V?
- Would it be predicted that single point mutation in a viral genome would have a dramatic impact on viral host tropism or disease pathogenesis? Why or why not?
- How does the CHIK-V E1 mutation and generation of the IOL strain compare with the emergence and pathogenesis of newly emergent influenza viruses?
- If Chikungunya has mainly been introduced to the United States through global travel, why are we concerned about increasing preventive efforts within this country?
- What are potential reasons for the spread of Chikungunya across the globe?
- What are potential reasons for the spread of Chikungunya to the United States?
- What are the implications for the increasing of rates of Chikungunya in the United States?

DIAGNOSIS OF CHIKUNGUNYA VIRUS IN PEOPLE

Chikungunya means “to walk bent over” in the Tanzanian language. The classic symptoms of CHIK-V are fever, back pain, headache and polyarthralgia with or without pruritic rash with desquamation of palms and soles and bullous facial edema. Because the symptoms are somewhat nonspecific, the differential diagnosis list may include a variety of viral, bacterial, and noninfectious causes. Therefore, other factors must be considered in development of the differential diagnosis list, most importantly the geographic location and travel history of the patient at the time of infection. Table 1 below delineates a fairly comprehensive differential diagnosis list for CHIK-V:

Table 1: Comprehensive Differential Diagnosis List for CHIK-V (endemic and non-endemic areas)

Acute Dengue fever	**Malaria**
Leptospirosis	**Parvovirus**
Measles	**Enterovirus**
Typhoid	**Adenovirus**
Rickettsia	**Other alphavirus infections such as West Nile virus and yellow fever virus**
Group A streptococcus	**Post-infectious arthritis**
Rubella	**Rheumatologic conditions**

Diagnosis of a patient with fever, myalgia, and polyarthralgia varies widely according to the patient’s geographic location and travel history. The diagnostic plan for the patient also changes significantly according to the socioeconomic status of the community, and the availability of advanced diagnostics in the region’s health care facilities. In general, if a patient presents with these symptoms and lived in a CHIK-V, malaria, and dengue-endemic area such as Central or South America, or Central or Southeast Asia, these three infectious diseases would be high on the differential diagnosis list. With a little bit more detail on clinical presentation using Table 2 below, the clinician could easily differentiate CHIK-V infection and acute Dengue fever without the use of advanced diagnostics unless the patient was unresponsive to symptomatic treatment for fever and arthralgia.

Tables 3 and 4 below help to further narrow the differential diagnosis list based on laboratory values. Fever and leukocytosis (over 5,000 cells/mm^3) accompanied by arthralgia and skin rash would more strongly suggest CHIK-V infection. On the other hand, the presence of neutropenia and thrombocytopenia with accompanying hemorrhage and hemoconcentration would increase likelihood of Dengue fever diagnosis; the lack of clinicopathologic laboratory abnormalities would be typical of a patient presenting with CHIK-V [28]. However in non-endemic areas, several other infectious/inflammatory diseases such as leptospirosis, malaria, typhoid fever, parvovirus, enterovirus, group A strep, rubella, measles, and alphaviruses such as West Nile virus, as well as several noninfectious etiologies (post-infectious arthritis and rheumatologic conditions) must also be considered, and advanced diagnostics may be required for a definitive diagnosis of CHIK-V.

Table 2: Comparison of Clinical Features of Chikungunya and Dengue Virus [adapted from Table 1, ref 16]

CLINICAL FEATURES	CHIK-V	Dengue Virus
Fever, asthenia	Common	Common
Myalgia	Common	Very common
Polyarthritis	Very common	Common
Tenosynovitis	Yes	None
Leukopenia	None	Yes
Thrombocytopenia	None	Yes
Rash	Days 1-4	Days 3-7
Retro-orbital pain	Rare	Common
Hypotension	Possible	Common
Second stage	Tenosynovitis, Raynaud's	Fatigue

Table 3: Analytes for 27 yo woman presenting with arthralgia, rash [adapted from Table 1, ref 29].

ANALYTE	PATIENT	REFERENCE VALUE
Hematocrit (%)	41.2	36.0-46.0
Hemoglobin (g/dL)	14.2	12.0-16.0
White cell count (per mm^3)	5600	4500-11,000
Neutrophils (%)	65.6	40-70
Lymphocytes (%)	21.6	22-44
Monocytes (%)	7.3	4-11
Eosinophils (%)	3.9	0-8
Basophils (%)	1.6	0-3
Platelet count (per mm^3)	107,000	150,000-400,000
Alanine aminotransferase (U/L)	52	7-33
Aspartate aminotransferase (U/L)	46	9-32

Table 4: Clinical Features of Chikungunya Virus Infection [adapted from Table 3, ref 29].

CLINICAL FEATURE	PREVALENCE IN CHIK-V PATIENTS, %		CLINICAL FEATURE PRESENT IN THIS PATIENT
	Returning travelers (n=20)	(During outbreak (n=157)	
Fever	100	96	YES
Rash	75	40	YES
Arthralgias	100	96	YES
Palpable Swelling	95	32	YES
Headache	20	47	YES
Nausea/vomit/diarrhea	15-30	47	YES
Conjunctivitis	20		YES
Pruritus	2		YES
Hemorrhage	5	6	YES (bleeding gums)
Leukopenia	25	79	NO
Thrombocytopenia	25	44	YES (mild)
Aminotransferase	25 elevated	2x upper limit in <10	YES (mild)
Creatine kinase		10	Not assessed
Hypocalcemia		55	NO

In a malaria-endemic area, fever accompanied by leukopenia and thrombocytopenia with or without a neutrophil left-shift without malarial prophylaxis would highly suggest malarial infection. Typhoid fever should also be suspected with fever and leukopenia without proper prophylaxis, whereas presence of macropapular rash and conjunctivitis may suggest leptospirosis [30].

These examples demonstrate two concepts:

1. Proper patient history, including recent travel, must be collected to aid in the process of diagnosis.
2. Worldwide, especially in medically underserved and poor socioeconomic areas, advanced diagnostics are not commonly used in the diagnosis of CHIK-V, as clinical features are more highly diagnostic of the disease than conventional bloodwork.

To address #1 above, the following questions must be posed of the patient during the process of attaining a patient history [30]:

1. Dates and locations of recent travel
2. Pre-travel prophylaxis
3. Exposures to insects or sick animals/people
4. Sexual activity with local people while traveling
5. Recent food and water exposures
6. Did the patient experience illness during travel and were any meds taken
7. Was the patient exposed to infectious agents after return from travel

Other key pieces of information, including timeline from exposure to onset of clinical signs, may also aid in the diagnosis of CHIK-V. For example, Table 5 lists approximate incubation times of infectious diseases that are high on the CHIK-V differential list:

Table 5: Incubation Periods of Common Infectious Diseases among Travelers Returning from the Caribbean or Central America [adapted from Table 2, ref 29].

DISEASE	MEAN INCUBATION PERIOD, days (range of days)
Chikungunya virus	2-4 (1-14)
Dengue	4-8 (3-14)
Malaria	6-30
Leptospirosis	5-14
Typhoid	7-18
Hepatitis A	28 (15-50)

Table 5 provides additional guidance in the diagnosis of CHIK-V and other insect-borne diseases, based on the length of incubation period. This information, along with presence of clinical signs, is critical in diagnosing CHIK-V in poor socioeconomic areas, where availability of advanced diagnostics is very limited.

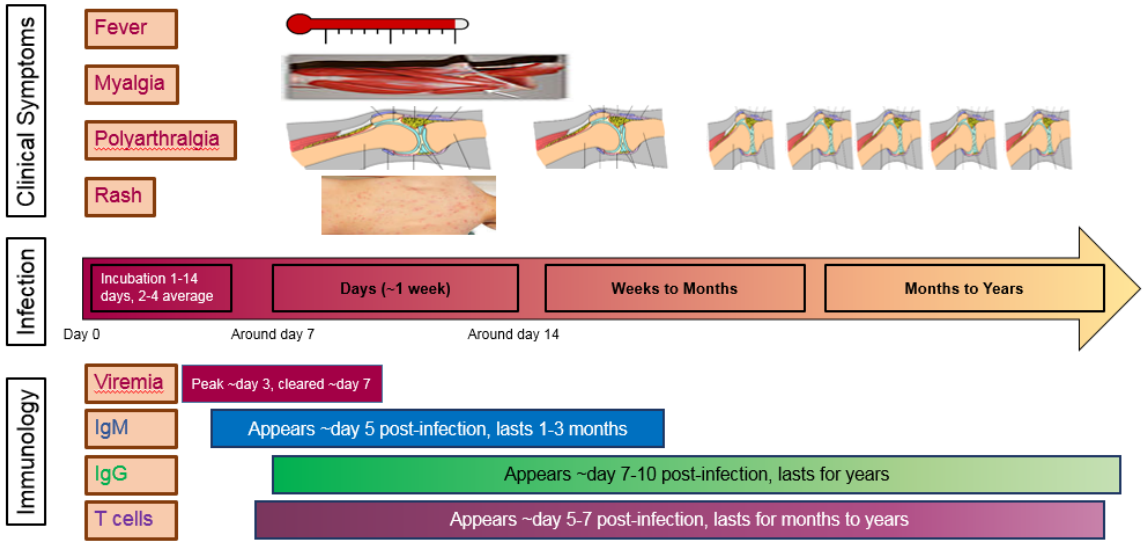

Figure 21. Timeline of Infection, Symptoms, and Biomarkers (adapted from [31]). This figure correlates the onset of clinical symptoms with the progression of viremia and humoral/cell-mediated immune response to the virus.

Given access to more advanced diagnostics, such as what would be available in developed countries' health care facilities, other tests may be performed that would help to utilize this information to predict the patient's course of disease:

- Blood smear with Giemsa stain which would positively diagnosis malaria
- Blood, urine, or fecal bacterial cultures for *Salmonella typhi (*typhoid fever).
- Serological tests (ELISA) to identify antigen-specific IgG or IgM.

These diagnostic tests would help to definitely diagnose CHIK-V, as antigen-specific IgM is typically present in serum of infected individuals beginning 5-7 days after mosquito bite, and is detectable for 3-6 months in the bloodstream; IgG is typically detectable in the serum of CHIK-V patients beginning at 7-10 days post infection and persists in the bloodstream for many months. Virus isolation can be performed 3 days post exposure, and PCR 7 days post exposure [32-34].

Diagnostic assays have been developed based upon the detection of IgM and IgG antibodies in the circulation specific for CHIK-V. These include both capture enzyme-linked immunosorbant assays (ELISA) and immunochromatographic tests. Importantly, it has been demonstrated that the currently available tests may not detect infection with the newly emerged CHIK-V variant containing the E1 A226V mutation [35]. Presumably, this mutation has significantly altered a major antigenic protein epitope for antibody production, changing antibody specificity in the immune response of the A226V-infected host such that antigens in the diagnostic test cannot be recognized by the patient's antibodies. Therefore, knowledge of the predominant CHIK-V isolates circulating in a specific geographic area should be known in order to accurately assess the reliability of these diagnostic assays and make the physician aware of the potential for false negative results.

Non-infectious rheumatologic disorders must also be considered in patients with polyarthralgia, especially in regions of the world where CHIK-V, dengue fever, leptospirosis, malaria, and typhoid are not endemic. The following rheumatologic disorders (rheumatoid arthritis, post-infectious arthritis, Lyme disease and lupus) would be diagnosed by: clinical presentation/ANA/dsDNA/anti-Smith antibodies (lupus); clinical signs such as ulnar deviation and phalangeal deformities and the presence of rheumatoid factor (rheumatoid arthritis); and clinical signs such as recent history of extraarticular infection (recent diarrhea, genitourinary infection, viral rash, etc.); and presence of neurological manifestations (polyradiculoneuritis, facial paralysis, encephalitis) concomitant with polyarthralgia in the case of Lyme disease [36-40]. Use of matrix-assisted laser desorption/ionization – time of flight tandem mass spectroscopy (MALDI-TOF MS-MS) for the identification of biomarkers of disease in blood/serum has the potential for immediate definitive diagnosis of such cases a reality in the very near future.

THE ROLE OF ANIMALS, AGRICULTURE, AND THE ENVIRONMENT IN THE SPREAD OF CHIKUNGUNYA VIRUS

There are a number of factors that may play a significant role in the spread of CHIK-V.

1. As previously described, ongoing changes in the weather patterns, as with El Nino rains, can create puddling and standing water that provide ample breeding grounds for mosquito vectors. This factor can be considered in rural, urban, and underserved areas of the world. Keeping in mind that this virus now has a new mosquito vector (*Ae. albopictus*) that has a broader geographic range, which provides greater opportunities for the virus to spread to more temperate climates.
2. Agricultural practices and animal husbandry also play an instrumental role in the spread of the virus: as farmers improve efficiency of their agricultural tasks, irrigation and moving livestock and domesticated animals closer to their dwelling places introduce breeding ground for mosquito vectors (water troughs and standing water), as above.
3. While this virus has traditionally demonstrated mosquito-human-mosquito transmission in its urban lifecycle, and active infection producing virus titers in the blood sufficient to allow transmission has only been demonstrated in bats and non-human primates, it appears domesticated animals can be exposed to the virus but are currently unable to support virus replication. However, with people living in closer proximity to their animals, there are increased opportunities for domesticated and other wild animals to be exposed to CHIK-V-infected mosquitoes. This virus has already demonstrated that small genetic mutations, as previously described in the attachment and fusion proteins, has had dramatic medical impact with spread of the disease to new geographic areas and making possible transmissible infection by a new mosquito vector. Thus, it is foreseeable that increased exposure to the virus in domesticated and other wild animals has the potential to exert sufficient evolutionary pressure on the virus, resulting in additional small genetic mutations that will enhance development of viremia and transmissible disease in these wild and domesticated animals that has been previously unreported.

Collectively, these factors clearly demonstrate the need for education and vigilance on the part of farmers, veterinarians, and public health officials as well as human health care workers to understand the pathogenesis, clinical signs, and significance of this disease. Keeping in mind that behaviorally, animals tend to mask symptoms of disease such as arthralgias and myalgias as a self-protective instinct, only astute observers would likely be able to identify animals who may begin to exhibit such clinical signs. Finally, effective communication between all members of the

one-health community, including human and animal health care providers, farmers, and environmentalists will be essential in identifying and controlling the spread and evolution of this disease in new geographic areas and new mammalian populations.

TREATMENT OF CHIKUNGUNYA FEVER IN HUMANS AND ANIMALS

As indicated in case #2 above, while the approach to diagnosis may vary according to geographic location of the patient, the treatment is the same in endemic and non-endemic areas, both for people and animals. Since there is presently no specific antiviral drug that is indicated in the treatment of Chikungunya fever, the treatment is typically limited to symptomatic care of the patient. Analgesics and nonsteroidal anti-inflammatory medications such as ibuprofen are typically recommended for relief of fever and joint pain. The critical factor in the management of CHIK-V is prevention and environmental control, in the form of removing standing water to eliminate breeding grounds for mosquitoes, and use of mosquito netting or barriers such as screens to reduce mosquito transmission. More information about preventing the spread of CHIK-V is included in the next section.

PREVENTING THE SPREAD OF CHIKUNGUNYA VIRUS

Environmental Control

The differences in cultural practices and living conditions between communities may contribute significantly to the risk of development of Chikungunya virus. The environmental, social, and biological determinants of health are described below, which relate to the following targets for intervention and prevention of the disease:

- Source reduction (e.g., removing stagnant water)
- Biocontrol (e.g. importing natural predators such as dragonflies, release of sterile males)
- Trapping and/or insecticides to kill larvae or adults
- Exclusion (mosquito nets and window screening)

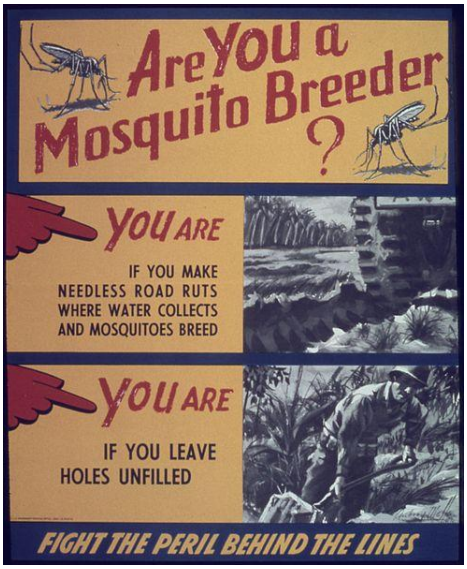

Figure 22. Are You a Mosquito Breeder? [41]. This is a poster that was created by the Office for Emergency Management. Office of War Information. Domestic Operations Branch. Bureau of Special Services. (03/09/1943 - 09/15/1945). This media is available in the holdings of the National Archives and Records Administration, cataloged under the ARC Identifier (National Archives Identifier) **513877**.

Source Reduction

Environmental factors are paramount to the spread of this disease: climate, climate change, and seasonal rainfalls are major determinants in the incidence of Chikungunya virus nationwide and worldwide. Additionally, local environmental factors such as the presence of standing water may encourage breeding of mosquito vectors and spread of the disease. Knowledge of the role of the environment is vital in the control of Chikungunya virus. Campaigns to reduce mosquito breeding have been used for over 60 years, as evidence in the World War II pamphlet depicted below. These informational campaigns were designed to target “source reduction”, which means elimination of breeding places of mosquitoes. It includes engineering measures such as filling, leveling and drainage of breeding places, and water management (such as intermittent irrigation). Source reduction can also include making water unsuitable for mosquito breeding (such as changing the salinity of the water if ecologically viable). Some specific measures are:

- For *Culex*: abolition of domestic and peridomestic sources of water suitable for breeding, for example removal and disposal of sewage and other waste water
- For *Aedes*: eliminating incidental containers such as discarded tins, crockery, pots, broken bottles, 55 gallon drums, dilapidated swimming pools, old bird basins, large puddles, coconut shells, or any outside object that may hold rainwater and maintaining clean water in outdoor containers for domesticated animals.

- For *Anopheles*: abolish breeding places by filling or drainage
- For *Mansonia*: removal of aquatic plants manually or by application of herbicides

Details of the biology of different species of mosquitoes differ too widely for any limited set of rules to be sufficient in all circumstances. However, the foregoing are the most economical/ecological and practical measures for most purposes. The importance of peridomestic control arises largely because most species of mosquitoes rarely travel more than a few hundred meters unless the wind is favorable.

Other forms of environmental control of the insect vectors are also being investigated, including:

Biological control or "biocontrol"
This term refers to the use of natural enemies to manage mosquito populations. There are several types of biological control methods including the direct introduction of non-ecologically invasive parasites, pathogens, vegetation, and predators (aquatic and non-aquatic) to target mosquitoes.

Various small fishes, such as species of *Galaxias* and members of the Poeciliidae, such as *Gambusia* (so-called mosquitofish) and guppies, and Banded killifish (*Fundulus diaphanus*), eat mosquito larvae and could be introduced into water sources for mosquito control. Other species of fish that have been shown to consume mosquito larvae are carp, minnows, bass, bluegill, piranhas, Arctic char, salmon, trout, catfish, goldfish, and tilapia [42]. Other predators such as dragonflies, lizards and geckos eat adult mosquitoes [43].

Other forms of biocontrol of mosquito populations include introducing large numbers of sterile males, complete eradication of certain mosquito populations that are known carriers of important diseases, genetic modification of mosquitoes, and use of the *thuringiensis* toxin which is a natural insecticide produced by the Gram positive rod *Bacillus thuringiensis*, a soil dwelling bacterium that has insecticidal properties against Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), Coleoptera (beetles), and Hymenoptera (wasps, bees, ants and sawflies). During sporulation the bacterium produces this endotoxin in crystal form, which is digested by the insect and then paralyzes the midgut of the insect, causing it to starve to death. The safety and efficacy of these control modalities in controlling the insect vector populations of several infectious diseases are presently under investigation to evaluate their safety and efficacy.

Trapping/Insecticides
Insect repellents are a second method to reduce mosquito bites. Repellants are topically applied on the skin to provide short-term protection against mosquito bites. Several chemicals are available, however the insecticide DEET has been shown to be more effective than others in repelling mosquitoes and other biting insects. Other repellants recommended by the CDC include: picaridin, eucalyptus oil, indalone, dimethyl phthalate, dimethyl carbate, and ethyl hexanediol. There are also electronic insect repellent devices that produce ultrasound and have been shown to repel insects (e.g., mosquitoes). However their effectiveness in reduction of disease prevention has not been demonstrated. There are commercial mosquito traps with mosquito attractants and source of carbon dioxide, which are also very effective in controlling mosquito populations around dwellings.

Exclusion
Social factors also play a significant role in the spread of Chikungunya virus. For example, those communities who live in open-air homes are at greater risk for mosquito bites; those who have standing water in outdoor containers are at greater risk for breeding mosquitoes; and travel and

economic trade also play a major role in the spread of the vector and the disease. In response to these social factors, there are several preventive measures to reduce the spread of this mosquito vector-borne disease, especially exclusion of the insects by using mosquito nets and window screens. In combination with scrupulous attention to control of breeding areas, window screens and mosquito nets are the most effective measures for residential areas. Insecticide-impregnated mosquito nets are particularly effective because they selectively kill those insects that attack humans, without affecting the general ecology of the area. An ideal mosquito net is white in color (to allow easy detection of mosquitoes), rectangular, netted on the sides and top, and without a hole. The size of the opening in net should not exceed 1.2 mm (0.047 in) in diameter, or about 23 holes per square centimeter (150 per square inch). Window screens should have copper or bronze gauze with 16 wires per inch.

Current Chikungunya Virus-Related Research

There are currently no vaccines or specific anti-viral drugs available for prevention or treatment of CHIK-V infection, and current therapy is limited non-specific, symptomatic treatments only as discussed above. However, CHIK-V has been designated an emerging disease with priority health and funding status by the National Institutes of Health. The rapid re-emergence and spread of CHIK-V and the NIH designation as a priority health challenge has spurred diverse research activities in the epidemiology, pathogenesis, control, and treatment of CHIK-V. Major topics of research include:

1) Epidemiology and Public Health
    i) Clinical attack rates and seroprevalence, particularly in Central America as disease is newly emerging there
    ii) The disease and economic burden of CHIK-V infection
    iii) The importance of field diagnosis for remote locations

2) Veterinary and Vector Factors
    i) Factors impacting virus infection and replication in *Aedes* mosquito vector
    ii) Use and efficacy of *Wolbachia* and *Bacillus thuringiensis* infection of mosquito vector in the inhibition of CHIK-V transmission
    iii) Development and comparison of lures, traps, and other mechanisms for the monitoring and control of *Aedes* vector
    iv) The effects of temperature and food (alone and in combination) on *Aedes* larva and adult mosquitoes and their ability to be infected by and to transmit CHIK-V
    v) Impact of the mosquito microbiome on acquisition and transmission of CHIK-V
3) Biomedical and Clinical
    i) Analyses of newly emergent and epidemic strains (sequencing, phylogentics, clade comparisons, relative in vitro infectivity comparisons)
    ii) Evaluation of CHIK-V disease manifestations – incidence, long-term outcome, clinical correlates
    iii) CHIK-V febrile illness – predisposing factors, incidence, outcome
    iv) CHIK-V-associated arthralgia – predisposing factors, incidence, outcome
    v) CHIK-V-associated encephalitis (thought to be under-diagnosed) – predisposing factors, incidence, outcome
    vi) CHIK-V-associated exanthems (thought to be under-diagnosed) – predisposing factors, incidence, outcome
    vii) Analyses of virus structure and its correlation and prediction of the protective capacity of neutralizing antibodies specific for CHIK-V or for alphaviruses
    viii) Identification of host susceptibility factors, especially those contributing to prolonged febrile illness and/or arthralgic disease

ix) Analysis of the host immune response to determine correlates of immune-protection and of immunopathology

4) Drug and Diagnostic Development
   i) Diagnostic assay development, particularly for use in rural/remote settings and for differentiation of Dengue virus infection
   ii) Prognostic value of musculoskeletal ultrasound in CHIK-V-associated arthralgias
   iii) Understanding the role of individual viral proteins in the infectious and pathogenic processes to identify potential targets of antiviral drug therapies
   iv) Identification and evaluation of CHIK-V and alphavirus-specific replication inhibitors
   v) Vaccine development – To date, two prophylactic vaccines have been evaluated in completed phase 1 safety clinical trials – a CHIK-V virus-like particle vaccine and a recombinant live-attenuated measles virus-vectored vaccine. Both are expected to progress to phase 2 clinical trials. Other vaccine strategies currently in preclinical development include a killed (formalin-inactivated) whole CHIK-V virus, a live-attenuated CHIK-V virus, DNA encoding CHIK-V antigens, and a CHIK-V E2 protein subunit vaccine.
   vi) Biologic immunotherapies – A phase 1/2 safety and efficacy clinical trial is currently underway to test the ability of hyper-immune sera to protect at-risk neonates against infection with Chikungunya virus. This therapy is aimed at the treatment of infants born in the midst of an outbreak to mothers who are currently experiencing CHIK-V viremia. It has been demonstrated that in outbreak settings, if the mother is viremic at the time of delivery, the incidence of mother-to-child CHIK-V transmission is 50%. In this study all infants infected at birth developed symptomatic disease. Furthermore, 50% of these infants progressed to severe forms of disease, predominantly due to damage to the central nervous system which resulted in permanent damage to the infant, leading to complications such as seizure disorders and cerebral palsy.

Questions for Students on the Diagnosis, Prevention and Control of Chikungunya

- When thinking about these two cases, why is it important to understand global differences between the burden of disease and how the disease presents in different cultural settings?

- What were the key symptoms described by the patient in case #1 that are associated with CHIK-V?

- What questions did Dr. Smith ask to rule out the differential diagnoses in case #1?

- What were the key questions that were influential in diagnosing the patient in case #1?

- What were the key lab results that confirmed the diagnosis?

- What other labs were performed to help establish the diagnosis?

- What was the treatment plan for the patient?

- How can the patient prevent future occurrences of CHIK-V?
- What follow-up is needed?
- What are the long-term effects of Chikungunya infection?
- How were the patient presentations similar between the two cases?
- What considerations led to the prompt diagnosis of the patient in El Salvador?
- What other conditions were the medical providers discussing with the patient in case #2 as a differential to her diagnosis? What were the warning signs to be aware of that she should recognize and know to return to be further assessed?
- Is the treatment of Chikungunya and are the methods of prevention any different from the patient seen in the U.S. and the patient seen in El Salvador?
- What are some of the major barriers to prevention that are occurring in regards to reducing the transmission of Chikungunya?
- What communication points do you think would be important to convey to the patient in the United States? In El Salvador?
- What preventive measures can be taken to prevent the transmission of Chikungunya virus?
- Do these measures differ for the U.S. population vs. The El Salvadoran population?
- What communication points do you think would be important for the veterinary community to address to pet owners, farmers who raise livestock, and those who live in rural environments and may encounter or live in close wild animals?
- What communication points would be vital to convey to other healthcare practitioners including nurses, health department personnel, etc.?
- Discuss the societal issues that must be considered with respect to the spread of CHIK-V to previously non-endemic areas, and what should the national priorities be with respect to public health education and control of the spread of this disease in first and third world countries?

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